RECEIVED
CENTRAL FAX CENTER

SEP 16 2004

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No.	:	unknown	Confirmation No. unkown
Applicant	:	Thomas D. Johnson	
Filed	:	September 13, 2004	
TG/A.U.	:	1651	
Examiner	:	Leon B. Lankford	
Docket No.	:	04-0910-JOHC	
Customer No.	:	26357	

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

NUMBER OF PAGES: 12 (Includes this page)

Certificate of Transmission

I hereby certify that this correspondence is being facsimile transmitted to the United States Patent and Trademark Office, Fax. No. 703-872-9306 on Sept 16, 2004

Typed name of person signing this certificate: Robert M. Hunter

Signature: Robert M. Hunter 42,679

PETITION UNDER 37 CFR § 5.12
Petition for license

Petitioner filed a parent application (10/067,185) on 02/01/2002. The parent application has been allowed and the issue fee payment was mailed on September 14, 2004. On September

09/20/2004 MAHMED1 00000030 10067185
01 FC:1460 130.00 OP

1/12

13, 2004, petitioner filed a U.S. CIP application adding the new matter (NM) enclosed with this petition. The new matter discloses other microorganisms that may be used in the invention disclosed in the parent application.

Petitioner desires to also file the CIP application in Canada before the parent U.S. application issues. Does petitioner need a foreign filing license in order to file the CIP in Canada? If so, how can an expedited foreign filing license be obtained?

If a foreign filing license is required, please fax a copy to 808-885-4114 or call 808-885-4194 as soon as possible.

A Credit Card payment form is attached for payment of the $130 fee.

Respectfully submitted,

Robert M. Hunter; Reg. No. 42,679; tel 808-885-4194; fax 808-885-4114
Address: P.O. Box 2709, Kamuela, HI 96743

2/12

TITLE OF THE INVENTION

CONTROLLING PLANT PATHOGENS WITH BACTERIAL/FUNGAL ANTAGONIST COMBINATIONS

Thomas D. Johnson

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of prior U.S. Patent Application No. 10/067,185, filed February 1, 2002, pending, which claims the benefit of U.S. Provisional Application No. 60/265,998, filed February 2, 2001; the disclosures of which applications are incorporated by reference as if fully set forth herein.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

The U.S. Government has a paid-up license in this invention and the right in limited circumstances to require the patent owner to license others on reasonable terms as provided for by the terms of Grant No. DMI-9901629 awarded by the National Science Foundation.

4/12

Working Examples presented herein. In fact, use of the antagonist combinations disclosed herein is shown to be functional when use of its individual constituent antagonists is not.

The compositions disclosed herein may be integrated into Integrated Pest Management (IPM) programs, the inventive compositions may be used in combination with other management systems. As an alternative to synthetic agents, biocontrol agents (bio-pesticides) offer the advantage of containing naturally derived constituents that are safe to both humans and the environment. Specifically, bio- pesticides offer such advantages as being inherently less toxic than conventional pesticides, generally affecting only the target pest and closely related organisms, and are often effective in very small quantities. For these reasons, bio-pesticides often decompose quickly and, therefore, are ideal for use as a component of Integrated Pest Management (IPM) programs.

The applicant has shown through a variety of laboratory and field trials that *Bacillus subtilis* var. *amyloliquefaciens* TJ 1000 and *Trichoderma virens* G1-3 are compatible with one another and that they act synergistically to consistently produce increased yield in plants. These results were presented in the parent application referenced above.

Field trials were conducted as part of the applicant's continuing research effort that tested other known *Bacillus subtilis* var. *amyloliquefaciens* (*Bacillus amyloliquefaciens*) strains and other known *Trichoderma virens* isolates. The purpose of testing was to determine whether the surprising synergism between a *Bacillus subtilis* var. *amyloliquefaciens* bacterium and a

NM

5/12

Trichoderma virens fungus disclosed in the parent application would be present between other strains and isolates of the same genus and species.

This testing by the applicant did result in the discovery of a synergistic activity between other isolates and strains of *Trichoderma virens* and *Bacillus subtilis* var. amyloliquefaciens. These results are presented in the final three working examples at the end of this document. The results show that other isolates of *Trichoderma virens* and other strains of *Bacillus subtilis* var. *amyloliquefaciens* do have synergistic properties. The applicant's research has also confirmed that the combination of *T. virens* G1-3 and *Bacillus subtilis* var. *amyloliquefaciens* TJ 1000 is superior to combinations comprising any other tested strains, but that synergies among other combinations do exist. These synergies have led the applicant to the conclusion that his patent rights should include combinations of all *Trichoderma virens* isolates and all *Bacillus subtilis* var. *amyloliquefaciens* strains.

NM

The invention is an inoculum, a seed coated with the inoculum, a plant protected with the inoculum, a method of producing the inoculum and a method of protecting a seed or a plant with the inoculum. A further embodiment of the inoculum comprises a combination of a fungus and a bacterium. Preferably, the fungus is a species of Trichoderma and the bacterium is a species of Bacillus, preferably a spore-forming strain of Bacillus. More preferably, the fungus is *Trichoderma virens* and the bacterium is *Bacillus subtilis* var. *amyloliquefaciens*, although other combinations are also envisioned. Even more preferably, the fungus is *Trichoderma virens* G1-3 (ATCC 58678) or *Trichoderma virens* Gl-21 (an isolate that is commercially available from Thermo Trilogy Corporation) and the bacterium is *Bacillus subtilis* var. *amyloliquefaciens*

6/12

Table 20. QuickRoots™ Effect on Corn Yield in Replicated Field Trials.

3 Year Average Evaluating QuickRoots™/Maxim vs. Maxim

Treatment	Moisture	Yield	Pricing	Advantage
Control	17.5	154.77	$300.25	
B. amyloliquefaciens alone	17.5	158.7	$307.88	$7.62
T. virens alone	17.4	158.81	$308.57	$8.31
B. amyloliquefaciens + *T. virens* combined 50/50	17.5	161.62	$313.54	$13.29
Mean	17.5	158.88	$307.56	
CV (%)	23.3	21.7		
LSD (0.05)	.19(NS)	5.05		

Corn Variety NK 2555 Treatment with Other Strains Working Example

Materials and Methods: For these studies *Trichoderma virens* Gl-21 (an isolate that is commercially available from Thermo Trilogy Corporation) and *Bacillus subtilis var. amyloliquefaciens* FZB24 (a strain that is commercially available from Earth Biosciences, Inc.) were selected. The plot entries (treatments) were as follows:

NM

7/12

all NM

Treatment A – Control (MAXIM, industry standard fungicide seed treatment)

Treatment B - *T. virens* G1-3 + *Bacillus subtilis* var. *amyloliquefaciens* TJ 1000

Treatment C - *T. virens* Gl-21 + *Bacillus subtilis* var. *amyloliquefaciens* TJ 1000

Treatment D - *T. virens* G1-3 + *Bacillus subtilis* var. *amyloliquefaciens* FZB24

Treatment E - *T. virens* Gl-21 + *Bacillus subtilis* var. *amyloliquefaciens* FZB24

The treatments were applied to corn seed (NK 2555) at equal rates of at least $1x10^6$ fungal spores and $1x10^6$ bacterial spores per seed. Previous field trials had confirmed that Treatment B produced an unexpected synergism that consistently and significantly increased yield in plants. The follow up field trials were conducted with the same test protocol as the initial trials and set up as a randomized – replicated block.

Results: Presented in Table 21 are the results of this trial. In this trial, all of the *T. virens* – *Bacillus subtilis* var. *amyloliquefaciens* combinations produced a numerically positive response. These results gave strong indication that combinations of *T. virens* and *Bacillus subtilis* var. *amyloliquefaciens* produce a synergistic effect that is similar to that discovered when *Trichoderma virens* G1-3 and *Bacillus subtilis* var. *amyloliquefaciens* TJ 1000 were combined and placed in the vicinity of the seed.

8/12

all NM

Table 21. Treatment of Corn Variety NK 2555 with Other Strains and Isolates

Treatment	Test Weight	Moisture	Yield
A	55.9	21.8	173.6
B	56.9	20.4	177.2
C	56.9	20.3	183.2
D	56.3	20.9	181.1
E	55.7	20.6	182.2
C.V.	5.4		
LSD .05	16.3		

Corn Variety NK 3030 Bt Treatment with Other Strains Working Example

This trial compared the treatment of *Trichoderma virens* G1-3 and *Bacillus subtilis* var. *amyloliquefaciens* TJ 1000 vs. *Trichoderma virens* GL-21 and *Bacillus subtilis* var. *amyloliquefaciens* FZB24 vs. a control (Maxim, industry standard fungicide seed treatment). Plot entries were as follows:

Treatment A – Control (MAXIM, industry standard fungicide seed treatment)

Treatment B - *T. virens G1-3* and *Bacillus subtilis* var. *amyloliquefaciens* TJ 1000

Treatment C – *T. virens Gl-21* and Bacillus *subtilis* var. *amyloliquefaciens* FZB24

9/12

all NM

Materials and Methods: Corn seed (NK 3030 Bt) was treated at the same rate of *Bacillus* and *Trichoderma* as in the previous working example and the seed was planted in a randomized – replicated block design.

Results: Presented in Table 22 are the results of this trial. In this trial, the yields of Treatments B and C were significantly greater than the control. Treatment B was numerically superior to Treatment C but not significantly. The results of this trial also indicated that other combinations of *T. virens* and *Bacillus subtilis* var. *amyloliquefaciens* can be expected to show a synergistic response.

Table 22. Treatment of Corn Variety NK 3030 Bt with Other Strains and Isolates

Treatment	Test Weight	Moisture	Yield
A	52.5	21.5	172.1
B	54.6	21.5	210.0
C	55.3	21.6	192.8
C.V.	8.09		
LSD .05	19.43		

Combined Trials with Other Strains Working Example

This example compared the same treatments as the previous working example, which were as follows: *Trichoderma virens* G1-3 and *Bacillus subtilis* var. *amyloliquefaciens* TJ 1000

10/12

all NM

vs. *Trichoderma virens* Gl-21 and *Bacillus subtilis* var. *amyloliquefaciens* FZB24 vs. a control (MAXIM). This trial differed from the previous working example because it compared 43 entries from 12 locations and 6 different corn hybrids. Plot entries were as follows:

Treatment A – Control (MAXIM, industry standard fungicide seed treatment)

Treatment B - *T. virens* G1-3 and *Bacillus subtilis* var. *amyloliquefaciens* TJ 1000

Treatment C – *T. virens* Gl-21 and *Bacillus subtilis* var. *amyloliquefaciens* FZB24

Materials and Methods: Seed was treated the same as in the previous two trials and each location was randomized and replicated.

Results: Presented in Table 23 are the results of this trial. This trial used a larger data set and revealed that the yield increase with the originally discovered combination of Treatment B (*Trichoderma virens* G1-3 and *Bacillus subtilis* var. *amyloliquefaciens* TJ 1000) is significantly greater than the control while the yield increase with Treatment C (*T. virens* Gl-21 and *Bacillus subtilis* var. *amyloliquefaciens* FZB24) is not significantly greater, even at the 0.20 rejection level. However, Treatment C did not show a numerical yield decrease nor did it show a significant yield decrease compared to the control. A yield decrease compared to the control would most likely have occurred if the microorganisms in the combination were antagonistic to each other. This result clearly showed that the original discovery (Treatment B) was superior to the Treatment C. The result also showed that Treatment C is a potentially beneficial treatment.

11/12

all NM

Table 23. Treatment with Other Strains and Isolates

Treatment		Yield in Bushels per Acre
A		153.84
B		160.63
C		156.36
C.V.	3.42	
LSD .20	4.4	

Many variations of the invention will occur to those skilled in the art. Some variations include non-competitive culturing of the biocontrol organisms. Other variations call for competitive culturing. All such variations are intended to be within the scope and spirit of the invention.

12/12